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Intraspecific Polyploids and Hybridization of *Plagiogyria adnata* and *P. yakushimensis* (Plagiogyriaceae)

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We report the somatic chromosome numbers of *Plagiogyria adnata*, *P. yakushimensis*, and a putative hybrid between them. The chromosome number of *P. adnata* was determined to be $2n = 260$ (tetraploid, $x = 65$) and $2n = 325$ (pentaploid, a new count). In *P. yakushimensis*, two cytotypes, $2n = 260$ (tetraploid) and $2n = 390$ (hexaploid), are reported for the first time. A pentaploid individual with $2n = c. 325$ is considered to be a hybrid between *P. adnata* and *P. yakusimensis*, based on cytology and leaf morphology. The present study indicates that sympatric populations of the two species have cytologically and morphologically diversified as a result of polyploidization and hybridization.

Key words: chromosome number, cytotype, hybrid, *Plagiogyria adnata*, *Plagiogyria yakushimensis*, Plagiogyriaceae, polyploid

Plagiogyria, the sole genus of the family Plagiogyriaceae, is distributed from Himalayas through Malesia, east to the Solomon Islands, north to Japan, and discontinuously in Central and South Americas (Zhang & Nooteboom 1998). *Plagiogyria adnata* (Blume) Bedd., an Asian species, is rather polymorphic and distributed widely in Asia from eastern India through China and Japan, and in Southeast Asia including western Malesia (Iwatsuki 1995, Zhang & Nooteboom 1998). The taxonomy of this species has been controversial, and Japanese populations have been variously treated. Copeland (1929), Tagawa (1939), DeVol (1972), Serizawa (1975), Iwatsuki (1995) and Zhang & Nooteboom (1998) treated Japanese populations as *P. adnata* in a broad sense, while Ching (1958, 1959) referred them to a new species, *P. distinctissima* Ching, and separated it from *P. adnata*. Nakaike (1971) and Dixit & Das (1981) recognized *P. rankanensis* Hayata originally described from Taiwan and reduced *P. distinctissima* to it, and Shieh *et al.* (1994) also adopted *P. rankanensis* for East Asian populations.

The polymorphism of *Plagiogyria adnata* includes dwarfism, which is seen in mountainous populations on Yakushima and Amami-oshima Isls., Kagoshima Prefecture, southern Japan. The dwarfs are characterized by small leaves (up to about 30 cm long *vs.* 70 cm in ordinary plants) and pinnae (up to about 4 cm long *vs.* 8 cm in ordinary plants), and, when dried, dark green laminae with conspicuous veins (*vs.* slightly yellowish green laminae with less conspicuous veins in ordinary plants). Sato (1936) first reported such a form from upland areas in Yakushima Isl. (1600-1700 m alt.) and described it as a distinct species, *Plagiogyria yakushimensis* Ka. Sato. This treatment was followed by Serizawa (1975). However, the taxonomic status of this species is controversial. The species was referred to *P. adnata* var. *yakushimensis* (Ka. Sato) Tagawa (Tagawa 1954, Yahara *et al.* 1987, Iwatsuki 1995) or *P. rankanensis* var. *yakushimensis* (Ka. Sato) Nakaike (Nakaike 1971). In contrast, *P. yakushimensis* was

reduced to *P. adnata* by Ito (1938) and Zhang & Nooteboom (1998), or to *P. rankanensis* by Dixit & Das (1981) and Nakaike (1992). On the other hand, Nakaike (1971) described another species, *Plagiogyria yakumonticola* Nakaike, from mountain forests on Yakushima Isl., while Serizawa (1975) and Yahara *et al.* (1987) assigned it to *P. yakushimensis* and *P. adnata* var. *yakushimensis*, respectively. DeVol (1972) also reported a dwarfed form of *P. adnata* from Pingtung County, Taiwan.

It is possible that the morphological variations of *Plagiogyria adnata* and *P. yakushimensis*, which are reflected by the various taxonomic treatments, are partly due to chromosomal variations. Cytological studies reported that materials from Yakushima Isl., Japan (under the name of *P. rankanensis*) and Chong-an County, Fujian, China (under the name of *P. distinctissima*) were $2n = 260$, a tetraploid of $x = 65$ (Nakato 1988), and $n = 60$, a tetraploid of $x = 30$ (Weng 1990), respectively. We report a chromosomal survey of *P. adnata*, *P. yakushimensis*, and a putative hybrid between them, and discuss cytological and morphological variations in sympatric populations of the two species. In the present paper, the nomenclature follows Serizawa (1975).

Materials and Methods

A list of materials, localities, and vouchers is given in Table 1. Materials were collected from forest floors along mountain paths on Kosugidani, Yakushima and Yuwandake, Amami-oshima Isls., Kagoshima Prefecture. In both collection sites, the two species grew side by side, and difference of habitat preferences was not recognized between them. For observations of somatic chromosomes, root tips were pretreated in 0.002 M 8-hydroxyquinoline solution for 6 hr at about 18°C. They were fixed in 45% acetic acid for 20 min, macerated with a mixture of 1N HCl and 45% acetic acid (4:1) at 60°C for 2 min, and squashed in 2% aceto-orcein. Vouchers are deposited in the Botanical Gardens, Graduate School of Science, University of Tokyo (TI).

Results

Results of chromosomal and morphological observations are listed in Table 1. Selected photographs of chromosomes are presented in Fig. 1, their explanatory drawings are shown in Fig. 2, and the silhouettes of some voucher specimens are in Fig. 3.

TABLE 1. Chromosome numbers and leaf and pinna sizes in *Plagiogyria adnata*, *P. yakushimensis* and their hybrid.

Taxon	Chromosome no. 2*n* (Ploidy)	Locality[1]	Voucher[2]	*N*[3]	Leaf length (cm)	Lamina length / Petiole length	No. of pinna pairs	Pinna Length × Width (cm)[4]
P. adnata	260 (4*x*)	Ama	*2341* (Fig. 3a)	3	52-55	0.9-1.0	15-16	7.0 × 1.2 - 7.3 × 1.2
	325 (5*x*)	Yak	*417* (Fig. 3b)	1	66	0.6	18	6.8 × 1.3
P. yakushimemsis	260 (4*x*)	Yak	*1760*	2	24-29	1.9	14-15	2.8 × 0.7 - 2.8 × 0.8
	260 (4*x*)	Yak	*1765* (Fig. 3d)	4	29-33	1.2-1.6	17-20	2.7 × 0.6 - 3.2 × 0.7
	260 (4*x*)	Ama	*2342* (Fig. 3f)	3	19-22	2.5-2.8	19-20	2.1 × 0.5 - 2.6 × 0.7
	c. 390 (6*x*)	Yak	*1761* (Fig. 3e)	2	31-32	1.5	18-19	3.7 × 0.7 - 4.4 × 0.8
	390 (6*x*)	Ama	*2340* (Fig. 3g)	3	22-25	2.2-2.5	18-19	2.7 × 0.7 - 3.2 × 0.8
P. adnata × *P. yakushimemsis*	*c.* 325 (5*x*)	Yak	*2296* (Fig. 3c)	5	40-49	1.7-2.3	19-20	4.9 × 1.1 - 5.8 × 1.1

[1] Abbreviations: Ama = Yuwandake, Amami-oshima Isl. ; Yak = Kosugidani, Yakushima Isl.

[2] Nakato's specimen no.

[3] Number of sterile leaves measured.

[4] The largest pinna of each leaf.

1. *Plagiogyria adnata*

An individual collected from Amami-oshima Isl. was found to be a tetraploid with $2n = 260$ based on $x = 65$ (Figs. 1a, 2a). The count is consistent with Nakato (1988) for a plant from Yakushima Isl. In contrast, one from Yakushima Isl. is a pentaploid with $2n = 325$ (Figs. 1b, 2b), the first count for the species.

In the tetraploid from Amami-oshima Isl., the sterile leaves are 52-55 cm long and the ratio of lamina/petiole length are 0.9-1.0 (Fig. 3a). In the pentaploid from Yakushima Isl., the leaf is 66 cm long and the ratio of lamina/petiole length is 0.6 (Fig. 3b). The lateral pinnae are 15- or 16-paired in the tetraploid and 18-paired in the pentaploid, and the largest pinna is 7.3 cm ×1.2 cm (length ×width) in the tetraploid and 6.8 cm ×1.3 cm in the pentaploid. Thus the two cytotypes were not distinguishable by gross leaf morphology.

2. *Plagiogyria yakushimensis*

Chromosome numbers were determined for five plants. Of these, two individuals from Yakushima Isl. and one from Amami-oshima Isl. are tetraploids with $2n = 260$ (Figs. 1c, 2c), while the other two, from Yakushima Isl. and Amami-oshima Isl., are hexaploids with $2n = c.\ 390$ and $2n = 390$, respectively (Figs. 1d, 1e, 2d, 2e).

In both cytotypes, the sterile leaves of voucher specimens from Yakushima Isl. are larger than those from Amami-oshima Isl. In the Yakushima's specimens, the sterile leaves are 24-33 cm long with 14-20 pairs of pinnae in the tetraploids, and 31-32 cm long with 18-19 pairs of pinnae in the hexaploid (Figs. 3d, 3e). The ratio of lamina/petiole length are 1.2-1.9 in the tetraploids and 1.5 in the hexaploid. The largest pinna is 2.8 cm ×0.8 cm or 3.2 cm ×0.7 cm in the tetraploids, and 4.4 cm ×0.8 cm in the hexaploid. In the plants from Amami-oshima Isl., the sterile leaves are 19-22 cm long with 19-20 pairs of pinnae in the tetraploid, and 22-25 cm long with 18-19 pairs of pinnae in the hexaploid (Figs. 3f, 3g). The ratio of lamina/petiole length are 2.5-2.8 in the tetraploid and 2.2-2.5 in the hexaploid. The largest pinna is 2.6 cm ×0.7 cm in the tetraploid and 3.2 cm ×0.8 cm in the hexaploid. The two cytotypes, therefore, could not be distinguished from one another by external morphology.

3. *Plagiogyria adnata* × *P. yakushimensis*

An individual from Yakushima Isl. was found to be a pentaploid with $2n = c.\ 325$ (Figs. 1f, 2f). The sterile leaves are 40-49 cm long, and the largest pinna is 5.8 cm ×1.1 cm, showing that it is intermediate between *P. adnata* and *P. yakushimensis* (Fig. 3c). The ratio of lamina/petiole length (1.7-2.3) is similar to those of *P. yakushimensis* (1.2-2.8), but the plant is distinguished from *P. yakushimensis* in that the leaves are yellowish green as in *P. adnata*. It is inferred that the plant is a hybrid between tetraploid *P. adnata* and hexaploid *P. yakushimensis*, or between hexaploid *P. adnata* and tetraploid *P. yakushimensis*, though hexaploid *P. adnata* has not yet been detected.

Discussion

The present study has revealed that different polyploid cytotypes occur in both *Plagiogyria adnata* ($4x$, $5x$) and *P. yakushimensis* ($4x$, $6x$). The pentaploid ($2n = 325$) and the hexaploid ($2n = 390$) are here recorded for the first time for *Plagiogyria*, and the latter is the highest chromosome number in Japanese leptosporangiate ferns (Nakato & Mitui 1983, Takamiya 1996). The origin of the pentaploid of *P. adnata* is uncertain, but it may have originated from crossing between a tetraploid and a hexaploid, although the latter has not yet been recorded. Furtheremore, it is inferred that the hexaploid of *P. yakushimensis* is derived either from a hybridization between a tetraploid and an octoploid, which has also not been detected, or from fertilization between a reduced diploid gamete and an unreduced tetraploid gamete of a tetraploid. Finding of tetraploids in the two species suggests that *P. yakushimensis* originated primarily at the tetraploid level from *P. adnata* or its related

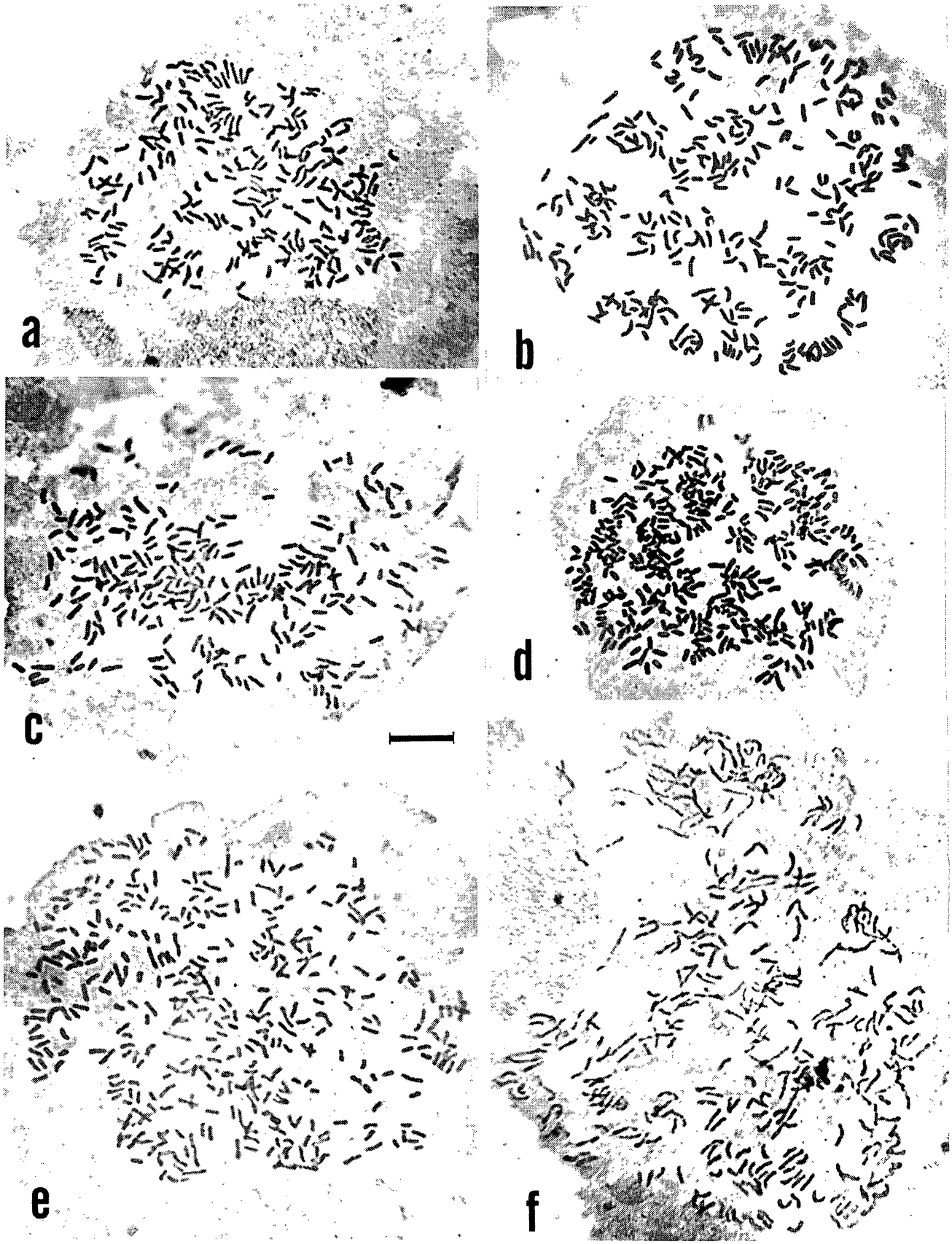

FIG. 1. Somatic chromosomes. a & b: *Plagiogyria adnata*. a: $2n = 260$, $4x$ (*no. 2341*). b: $2n = 325$, $5x$ (*no. 417*). c-e: *P. yakushimensis*. c: $2n = 260$, $4x$ (*no. 2342*). d: $2n = c.\ 390$, $6x$ (*no. 1761*). e: $2n = 390$, $6x$ (*no. 2340*). f: *P. adnata* × *P. yakushimensis*. $2n = c.\ 325$, $5x$ (*no. 2296*). Scale bar = 10 μm.

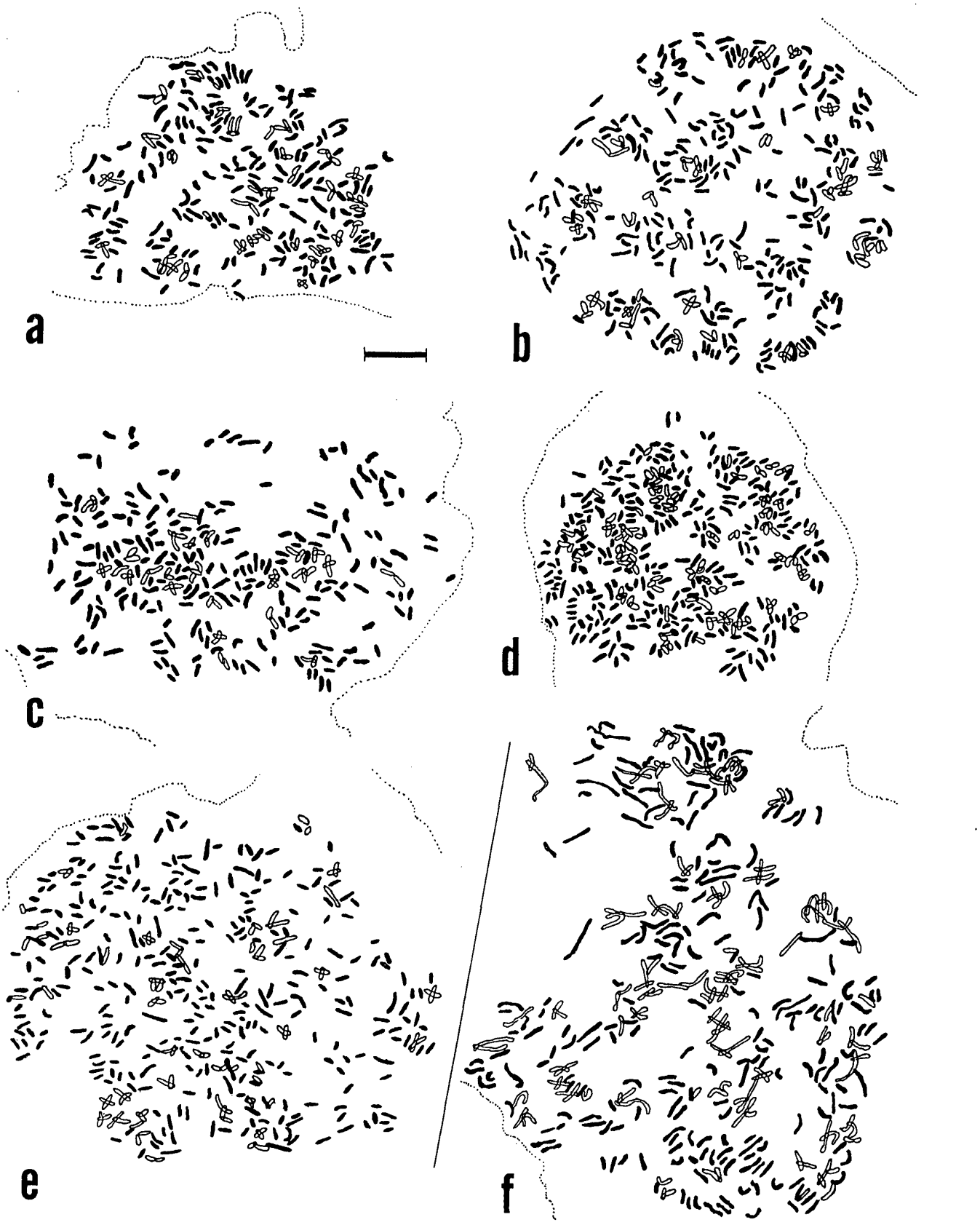

FIG. 2. Explanatory drawings of Fig. 1. Chromosomes overlapped are shown in outline.

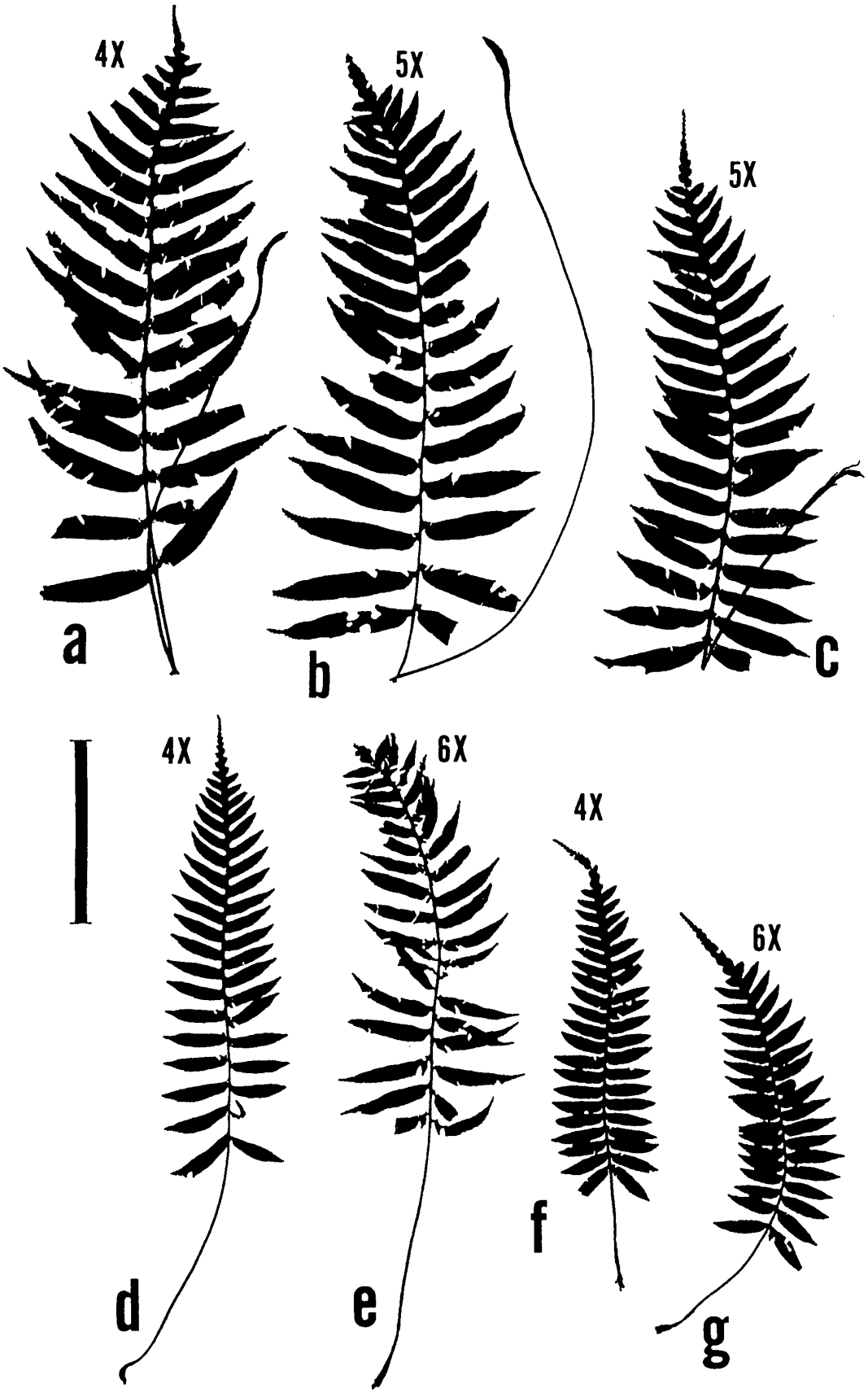

FIG. 3. Silhouettes of sterile leaves of cytological voucher specimens. a & b: *Plagiogyria adnata*. a: *no. 2341*. b: *no. 417*. c: *P. adnata* × *P. yakushimensis*, *no. 2296*. d-g: *P. yakushimensis*.d: *no. 1765*. e: *no. 1761*. f: *no. 2342*. g: *no. 2340*. Scale bar = 10 cm.

species.

The taxonomic status of *Plagiogyria yakushimensis* has been controversial. This seems to be mainly due to the occurrence of various intermediate forms between *P. adnata* and *P. yakushimensis*. The pentaploid hybrid between the two species reported here is actually such an intermediate form. In spite of only a limited number of plants examined, it appears that sympatric populations of the two species are cytologically and morphologically diversified as a result of hybridization as well as polyploidization. A tetraploid hybrid, *P. adnata* (4*x*) × *P. yakushimensis* (4*x*), may be found in further investigations. The fertility of the hybrid, if found, may suggest whether the two species are distinct or conspecific.

Nakaike (1971) described plants that are similar to *Plagiogyria yakushimensis* as *P. yakumonticola*. The species was characterized as follows: the lateral pinnae about 20-paired, the lowest 2-3 pinnae reflexed, and the basal pinnae hardly decurrent on the acroscopic side. In comparison, the type specimen of *P. yakushimensis* has 11-15 pairs of lateral pinnae and reflexed lowest 1-2 pinnae decurrent on acroscopic side (Sato 1936). The tetraploid of the *P. yakushimensis* complex examined have 14-20 pairs of pinnae and the hexaploids have 18-19 pairs of pinnae, and both cytotypes have reflexed 1-3 pairs of basal pinnae. A few voucher specimens show intermediate characters between *P. yakushimensis* and *P. yakumonticola*. For example, the tetraploid specimen, *no. 1765*, have 17-20 pairs of pinnae, and reflexed lowest 1-3 pinnae, most of which are decurrent on the acroscopic side. Therefore, *P. yakumonticola* seems not to be distinguished from *P. yakushimensis*, as noted by Serizawa (1975).

Weng (1990) reported Chinese *Plagiogyria distinctissima* to have $n = 60$ and interpreted it as a tetraploid based on $x = 30$. Although this species was recently reduced to *P. adnata* (Zhang & Nooteboom 1998), the two have been enumerated as independent species in some Floras published in China (see Ching 1959, Kung 1988, Lin *et al.* 1991, Zhang 1993). It seems to us that Weng's (1990, plate 3) photograph of a spore mother cell shows more than 60 bivalent chromosomes, so the species needs further cytotaxonomic research. However, the ploidy level of the Weng's specimen is obviously diploid. No diploid has been recorded in the Japanese *P. adnata* and *P. yakushimensis*.

Although various base numbers have been proposed for *Palgiogyria*, they are probably $x = 65$ and $x = 66$ (Nakato & Mitui 1983). Diploid species in this genus include *P. semicordata* (C. Presl) H. Christ ($n = 66$) from Jamaica, *P. tuberculata* Copel. ($n = 66$) from New Guinea and *P. matsumureana* Makino ($n = 65$, $2n = 130$) from Japan (Walker 1966, 1973, Nakato & Mitui 1983) as well as possibly *P. distinctissima*. Moreover, Taiwanese *P. formosana* Nakai ($n = 75$) and *P. stenoptera* (Hance) Diels ($n = 75$) may be diploid (Tsai 1973), though the recorded number of $n = 75$ needs to be reexamined (see Nakato & Mitui 1983). Except for *P. adnata* and *P. yakushimensis*, tetraploid chromosome numbers were reported in *P. glauca* (Blume) Mett. ($n = c.132$) from Java, *P. euphlebia* (Kunze) Mett. ($n = c.130$, $2n = 260$) and *P. japonica* Nakai ($2n = 260$) from Japan (Walker 1973, Nakato & Mitui 1983). As a result, available chromosomal information suggests that polyploidization plays an important role for diversification and speciation in *Plagiogyria*, particularly in *P. adnata* and its allied taxa. To investigate the systematics of the *P. adnata* group, it is necessary to make further extensive cytotaxonomic research on plants from a wide distribution range.

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